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Seminar 4: How to set up a calibration Table in ChemStation

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Quantification - Calibration
In this Section, We Will Discuss:

The steps necessary to build a calibration table.

How to select calibration settings.

Calibration table options.

Peak identification tools.
Building a Calibration Table

1. Inject standards with concentrations that bracket your unknowns.

2. Set-up appropriate integration events for the low standard. Integrate to obtain the peak height or area. Save the integration events to the method.

3. Begin a new calibration table with level one, the lowest level. Fill in compound names and amounts.

4. Integrate each additional standard level and add to the calibration table.

5. Examine the calibration curve and save it to the method.
Example Calibration

3 Levels

\[
\begin{align*}
1. \text{ Standard} & \quad 30.0 \quad \text{ISTD} & \quad 50.0 \\
2. \text{ Standard} & \quad 50.0 \quad \text{ISTD} & \quad 50.0 \\
3. \text{ Standard} & \quad 75.0 \quad \text{ISTD} & \quad 50.0
\end{align*}
\]
Signal Details

Define signals to be evaluated during a method.
Calibration Settings

Data Analysis:
- Calibration
- Report
- Spectra
- Bio
  - New Calibration Table...
  - Delete Calibration Table...
  - Recalibrate...
  - Add Level...
  - Add Peaks...
- Calibration Settings...
  - Advanced Calibration
  - Calibration Table Options...
- Select Peak
- Delete Peaks
- Add Peaks
- Recalibrate Compounds
- Calibration Table...
- Compound Groups...
- Signal Details...
- Control Sample Limits...

Calibration Settings: Instrument 1
- Title
- Use Sample Data: From Data File
- Sample Defaults: From Sample Defaults Below
- From Data File
- Amount: 0.000
- Amount Units: ng/ul
- Multiplier: 1.000
- Dilution: 1.000

Default RT Windows:
- Reference Peaks: 0.00 + 5.00
- Other Peaks: 0.00 + 5.00

Default Calibration Curve:
- Type: Linear
- Origin: Include
- Weight: Equal

Calculate Uncalibrated Peaks:
- For Signal: DAD1 A, Sig=254.4 Ref=off
- Using Compound: None
- With Rsp Factor: 0.000
- Use ISTD: None
- No

ISTD Correction:
- Use Multiplier & Dilution Factor with ISTDs

OK  Cancel  Help
Load the Signal(s) for the Low Level Standard and Integrate
Create a Calibration Table: Level One

![Calibration Table Interface]

**Calibrate: Instrument 1**

- **New Calibration Table**
  - Manual Setup
  - Automatic Setup Level: 1
  - Default Amount: 0.000

**Calibration Mode**
- Calculate Signals Separately

**Data Analysis**
- Calibration
- Report
- Spectra
- Base
- New Calibration Table
  - Delete Calibration Table
  - Recalibrate
  - Add Level
  - Add Peaks
- Calibration Settings
  - Advanced Calibration
- Calibration Table Options
  - Select Peak
  - Delete Peaks
  - Add Peaks
  - Recalibrate Compounds
- Calibration Table
  - Compound Groups
  - Signal Details
  - Control Sample Limits
Calibration Table Overview: Fill in the Dialog

Fill in:
- Compound Name
- Amount

Check if:
- Reference Peak
- ISTD

Indicate ISTD #
Demo Calibration
Load the Signal - Add Level Two

Fill in Amount for Level 2
Add Additional Levels

Add Level 3 and fill in the amount
Calibration Table Options

- Compound, Amt, Area, Rsp Factor, Ref, ISTD#
- Compound, Grp, Amt, Low Limit, High Limit
- Compound, Amt, Area, Def, Curve Type, Origin, Weight
- Compound, Area, Rsp%, Def, +-, Pk Usage
Use Low and High Limits as qualifiers to confirm peak identification.
Peak Details

Check **Def** to apply the default curve type and origin from the Calibration Settings dialog box.

### Curve Types
- Piece wise
- Linear
- Log
- Power

### Origin Treatments
- Exponent
- Quadratic
- Cubic
- Average Rsp/Amt
- Ignore
- Include
- Force
- Connect straight segment
By default, the largest peak of a compound is the Main peak. Others are set to Ignore.
Qualifiers

The ratio of 230 to 254 is 38% for Biphenyl

The ratio of 230 to 254 is 224%. The peak would be rejected
Define the columns included in your Calibration Table
Quantification Tools

New Calibration Table

Calibration Task Tool

Add New Level

Add New Peaks

Signal Details

Edit Calibration Table Options

Recalibrate with Current Chromatogram

Select Calibrated peak(s) from Chromatogram and delete from calibration table.

Select peak(s) from chromatogram and add to calibration table

Select compounds from chromatogram and recalibrate
To Save the Calibration Table as Part of a Method...

Save Table to Method
Save Method

- Be sure to know which method you are working with and save changes to the correct method.

- Method Development changes (i.e. calibration table creation / updates) should be saved to the Current Method (Master Method).

- Integration events and other parameters specific to the individual data file should be saved to the Individual Method from Data File (DA.M)
Information on ChemStation

Documentation can be found on the Agilent website
- Product Datasheet
- Specification
- Application Notes
- Manuals

Manuals can be found your ChemStation Installation DVD
- Getting Started with New ChemStation Workflow
  PartNo.G2170-90042
- Understanding your ChemStation
  PartNo. G2070-91125
- OpenLAB Option
  PartNo.G2170-90233

Software Status Bulletin

Customer Trainings (NorthAmerica)
Learning Products – North America Course Catalog

Users needing to increase productivity in the lab by utilizing both standard and advanced features available in the Agilent GC/LC ChemStation may want to attend one of the following courses:

- H2606A - ChemStation for GC Data Analysis and Reporting (2 days)
- H5928A - Agilent HPLC (2D) Data Analysis and Reporting (2 days)
- H4039A - Agilent HPLC (3D) Data Analysis and Reporting (3 days)

Course Features

- Data acquisition and method creation
- Data analysis including integration and calibration
- Sequencing
- Reporting

For more information concerning course content, dates and locations, please visit:
Users needing to increase productivity in the lab by utilizing both standard and advanced features available in the Agilent GC/LC ChemStation may want to attend one of the following courses:

- H4033A – Agilent HPLC (3D) Method & Run Control, Data Analysis and Reporting (4 days)
- H8718A – Agilent HPLC (3D) Data Analysis and Reporting (2 days)
- H5928A – Advanced User Training, Quantification and Result Reporting (2 days)

**Course Features**

- Data acquisition and method creation
- Data analysis including integration and calibration
- Sequencing
- Reporting

For more information concerning course content, dates and locations, please visit:

Who to contact for more information about OpenLAB and Agilent software products

1. Agilent Account Managers (sales)

2. Agilent Informatics Product Specialists

   Eastern US - Marc Mandelbaum – 302-683-7149
   Pacific Coast (CA, WA, OR) - Spencer Tse  408-553-3655
   Midwest and South - Johnathon McSayles  847-944-6019
   Midwest /West/Latin America – Stephen Brown 847-944-6461
QUESTIONS?